A new mechanism for bile acid diarrhea: excessive bile acid biosynthesis caused by defective fibroblast growth factor 19 release from the ileum

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FGF19 in bile acid malabsorption

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Abbreviations:
BAM, bile acid malabsorption; FGF19, Fibroblast Growth Factor 19; C4, 7α-hydroxy-4-cholesten-3-one; SeHCAT, selenium homocholic acid taurine; ASBT, apical sodium-dependent bile acid transporter; FXR, farnesoid X receptor

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ABSTRACT:

Background & Aims: Primary (idiopathic) bile acid malabsorption (BAM) is a common chronic diarrheal syndrome which is under-recognized as diagnosis is difficult without selenium homocholic acid taurine (SeHCAT) testing. Diarrhea results from excess colonic bile acids producing secretion, but the pathogenesis is unclear. Fibroblast growth factor 19 (FGF19), produced in the ileum in response to bile acid absorption, has been shown to regulate hepatic bile acid synthesis. We hypothesized FGF19 could be involved in bile acid diarrhea and measured this in patients with BAM.

Patients and Methods: Blood was collected from fasting patients with chronic diarrhea where BAM was diagnosed by SeHCAT. Serum FGF19 levels were measured by ELISAs. Serum 7α-hydroxy-4-cholesten-3-one (C4) levels were determined using HPLC, to quantify bile acid synthesis. Data were compared between patients and subjects without diarrhea (controls). Repeated samples were taken after meals from several subjects.

Results: The median C4 level was significantly higher patients with primary BAM than controls (51 vs 18 ng/ml; $p<0.0001$); the median FGF19 level was significantly lower in patients with BAM (120 vs 231 pg/ml; $p<0.0005$). There was a significant inverse relationship between FGF19 and C4 levels ($p<0.0004$). Low levels of FGF19 were also found in patients with post-cholecystectomy and secondary bile acid diarrhea. Abnormal patterns of FGF19 levels were observed throughout the day in some patients with primary BAM.

Conclusions: Patients with BAM have reduced serum levels of FGF19; this finding might be useful in diagnosis. We propose a mechanism whereby impaired FGF19 feedback inhibition causes excessive bile acid synthesis that exceeds the normal capacity for ileal reabsorption, producing bile acid diarrhea.

Keywords:
Diarrheal diseases, Bile acids, Enterohepatic circulation, Ileum, Irritable bowel syndrome

INTRODUCTION

Chronic watery diarrhea can be produced by the stimulation of water secretion in the colon by high concentrations of bile acids. This condition is called bile acid malabsorption (BAM), and responds to treatment with bile acid sequestrants such as cholestyramine, colestipol or colesevelam. [1-3]

Bile acids are usually reabsorbed efficiently in the terminal ileum, and undergo an enterohepatic circulation where they are resecreted by the liver. Although the total bile acid pool is quite variable, the mean value has been measured at about 5 mmol (2g) in a 70kg man, with total hepatic secretion up to 30mmol (12g) in 24h. This indicates the importance of absorption and resecretion, with recycling estimated to average 4 to 6 times a day, depending in part on diet [4]. Surgical resection of the terminal ileum, or inflammation as in Crohn's disease is well-recognized as producing the condition known as secondary bile acid malabsorption (or type 1 BAM), with clear impairment of reabsorption, and bile acid diarrhea.

Bile acid malabsorption can be diagnosed relatively easily by the SeHCAT (selenium homocholic acid taurine) test, where a 7-day retention of ^{75}SeHCAT of less than 10-15% is abnormal. [5, 6] This test is available in most European countries but is unavailable in the USA which has hindered the recognition of the importance of this condition. Radiolabelled glycocholate has been used previously to diagnose this condition. Ideally, measurement of fecal bile acid output is needed, although this is not readily available outside of clinical investigation units and is inconvenient for patients. When bile acids are lost, a compensatory increase in activity of hepatic cholesterol 7α hydroxylase (CYP7A1) produces increased bile acid synthesis (chenodeoxycholic and cholic acid). The increase in the serum levels of the bile acid precursor, 7α-hydroxy-4-cholesten-3-one (C4), can be measured to diagnose bile acid malabsorption. [7]

Primary, or idiopathic, bile acid malabsorption (primary BAM) is the condition where there is chronic diarrhea, low SeHCAT retention, raised C4 and a response to bile acid sequestrants, but where there is no resection or obvious disease of the ileum [8, 9]. This condition, also known

as type 2 BAM, is detected by the SeHCAT test, and where this is performed, has been found to be relatively prevalent. Many reports, mostly from Europe, indicate primary BAM can account for around 30% of patients with chronic diarrhea, who might otherwise be diagnosed as having diarrhea-predominant irritable bowel syndrome (IBS)[10-15]. Bile acid malabsorption can also be found in various other gastro-intestinal conditions (type 3), including post-cholecystectomy where the cause is presumed to be related to intestinal absorption not being able to compensate for the disordered temporal pattern of bile secretion [16].

Surprisingly, the available evidence suggests that there is no defect in ileal bile acid transport in the majority of patients with primary BAM. Although a mutation in the gene coding for apical sodium-dependent bile acid transporter (ASBT) was shown in one family [17], this is not present in most patients [18]. Normal uptake of bile acids into ileal brush-border membranes [19] and into intact mucosal biopsies has been shown in primary BAM [20], and we have recently found no significant difference in ileal expression levels of transcripts for the transport proteins, including ASBT, the cytoplasmic ileal bile acid binding protein and the basolateral organic solute transporters [21].

These findings suggest other mechanisms may be responsible for primary BAM. More rapid small bowel transit, or a reduced length of small intestine able to transport bile acids have been suggested [20-22]. Disorders in the regulation of the enterohepatic circulation could also be causative, and studying this represents a new approach to the pathophysiology of this disorder.

Ileal enterocytes synthesize Fibroblast Growth Factor 19 (FGF19) in humans [21] and the circulating protein has been demonstrated to have a diurnal variation related to bile acid synthesis [23]. The orthologue of human FGF19 in mice is FGF15. This has been shown to inhibit bile acid synthesis in the liver, acting through the FGFR4 receptor [24]. FGF15 knock-out mice have a condition which resembles primary BAM, with increased levels of hepatic synthesis and faecal bile acid secretion.

We hypothesize that the clinical picture of primary BAM could result from disordered signaling by FGF19. We have measured serum levels of FGF19 in patients with bile acid malabsorption and in controls, and related this to bile acid synthesis.

METHODS

Subjects

Patients with chronic diarrhea were identified in Gastroenterology out-patient clinics and gave informed consent. The study was approved by the local Institutional Review Board, the Hammersmith, Queen Charlotte's & Chelsea Hospitals Research Ethics Committee. All 17 patients had troublesome watery diarrhea with more than three stools per day for more than three months. Other causes of diarrhea including Crohn's disease, coeliac disease, lactose intolerance and short bowel syndrome had been excluded. SeHCAT testing was performed in 13 patients: all had less than 8% retention at 7 days. All patients had at least a partial response to bile acid sequestrants. Five patients with diarrhea had undergone cholecystectomy which had aggravated their symptoms; 4 of these had undergone SeHCAT tests, which were all abnormal. Fasting blood was collected, and the serum separated and stored frozen at -20 °C prior to assay. Bile acid sequestrants were discontinued at least 24 h before the blood samples were obtained.

Control subjects were recruited from patients without diarrhea or liver disease, and from healthy staff. No control had a bowel frequency greater than twice daily. Additionally, fasting blood samples were obtained from a separate comparison group of patients with diarrhea and bile acid malabsorption secondary to ileal resection. The control group and the group with ileal resection did not undergo SeHCAT testing.

Six patients (5 Females, 1 Male), with SeHCAT results ranging from 0-5%, had repeated blood samples taken every 90 minutes throughout the day to see whether there were any changes in the diurnal patterns of C4 and FGF19 secretion. The first sample was taken after an overnight fast at 9.00 am and was followed by breakfast. Lunch was eaten after the third sample (12 noon). No bile acid sequestrants were taken during the previous 24 hours.

Laboratory assays

Serum levels of FGF19 were measured by ELISA (FGF19 Quantikine ELISA kit, Cat. No. DF1900; R&D Systems, Minneapolis, MN, USA), according to the manufacturer's protocols.

Serum 7α-hydroxy-4-cholesten-3-one (C4) was determined using HPLC following solid phase extraction (SPE) as previously described [7]. Total serum bile acids were measured by an enzymatic colorimetric method using 3-alpha hydroxysteroid dehydrogenase (ref. 6K9001; Sentinel Diagnostics, Milan, Italy).

Statistics

Statistical analyses were performed with Winstat for Excel, using non-parametric testing. Mann-Whitney U-tests and Spearman rank correlation tests were used as appropriate.

RESULTS

The details of the patient group and the controls are shown in Table 1. As expected, there was a highly significant difference in bowel frequency. Fasting serum C4 values were significantly higher in the patient group than in controls (median 51 vs. 18 ng/ml; $p < 0.0001$, Mann-Whitney U-test) (Fig. 1a).

FGF19 was detected in the serum of all the patients and controls, with fasting values ranging from 16 to 655 pg/ml. Fasting serum FGF19 values were significantly lower ($p = 0.0005$) in the patients (median 120 pg/ml, range 16–364) than in controls (231 pg/ml, 74–655 pg/ml) (Fig. 1b). Overall, FGF19 and C4 were inversely related ($r = -0.54$; $p < 0.0004$, Spearman rank) (Fig. 2). Within the groups, the inverse relationship was found in patients ($r=-0.53$, $p=0.01$) but was not significant in controls ($r=0.06$). The product of C4 and FGF19 was significantly higher (an increase of 75% in the median) in the patient group than in the controls ($p<0.05$) implying a different homeostatic equilibrium. FGF19 levels were not related to previous use of bile acid sequestrants.

We also analyzed the findings in the subgroup of 13 patients who had undergone SeHCAT testing (Table 2). The FGF19 median was lower than in the overall group, and range of values was smaller. We further subdivided this group according to whether they had undergone a cholecystectomy, and both groups had significantly lower FGF19 median values than controls. The subgroup with a low SeHCAT test, and who had not undergone a cholecystectomy (that is those fitting the definition of type 2 BAM), all had fasting FGF19 values below 146 pg/ml. Only 2 of the 19 controls were below this value (74 and 140 pg/ml). The median value in this subgroup with primary BAM was highly significantly lower than the control group ($p=0.0001$).

For comparison we also studied 5 patients who had diarrhea due to bile acid malabsorption secondary to previous ileal surgery (type 1 BAM). As shown in Table 2, these also had significantly raised C4 and low FGF19 values, with very low values (<10pg/ml) in some patients with extensive resection.

Serum total bile acids (Table 2) were not significantly different between the overall patient and control groups, although the median in patients with abnormal SeHCAT and no cholecystectomy was higher. In the group with secondary bile acid malabsorption, the median bile acid value was lower, but neither difference reached significance.

The changes in FGF19 and C4 throughout the day produced variable patterns (Fig. 3). In some patients, a pattern resembling that reported in normal subjects was found, with a rise in FGF19 occurring after lunch [23]. Two subjects had no changes in FGF19 throughout the day, which is a fasting pattern, despite taking breakfast and lunch and having an elevation in bile acids (not shown).

DISCUSSION

This is the first study to our knowledge where serum FGF19 has been investigated in patients with bile acid malabsorption. We found reduced levels of FGF19 in patients, associated with increased C4, indicating increased bile acid synthesis. These changes were present in the patients irrespective of whether their symptoms were associated with previous bowel resection or cholecystectomy, or whether they appeared to have primary BAM.

In our patients with primary BAM, serum bile acids were higher than in controls; although this did not reach significance, it led us to consider whether an increase in bile acid production could be a potential cause of this condition. Review of the literature showed that there is no simple relationship between serum bile acid levels, bile acid pool size, bile acid secretion and bile acid biosynthesis rate, but when the bile acid pool was calculated in patients similar to ours by Van Tilburg *et al.* in 1992 [25], a significant increase of about 90% was found with a mean of 7.0 mmol vs. 3.7 in controls. Considering the recent work that has failed to show any defect of bile acid uptake into ileal mucosal biopsies [20], this would indicate an alternative mechanism whereby defective negative feedback regulation of bile acid synthesis by FGF19 could be the central feature that produces this syndrome.

FGF15 is orthologous in mice with FGF19 in humans, and was identified as an enterohepatic signal produced by the ileum which was critical to the regulation of bile acid homeostasis [24]. Previously, in studies of agonists of the bile acid receptor FXR, FGF19 was shown to be markedly stimulated [26]. Mice where FGF15, or its receptor, FGFR4, have been deleted, have watery diarrhea resembling that associated with primary BAM [27]. FGF15 was shown to reduce the diarrhea in Asbt-null mice [28]. Additionally FGF15 has roles in gall bladder relaxation [29]. Studies in man which relate FGF19 to bile acid synthesis have shown evidence of diurnal changes, an increase in FGF19 with supplemental bile acids, and a reduction with sequestrants [23].

According to the model we are proposing (Fig. 4), reduced FGF19 synthesis from the ileum would result in an impaired feedback inhibition of CYP7A1 in the liver, and consequently

an increase in bile acid synthesis. This would be reflected in the raised C4 levels. Excess bile acids produced by the liver and secreted into the small intestine would exceed the capacity for reabsorption in the otherwise normal ileum, and so an increased amount of bile acids will enter the colon and produce diarrhea. SeHCAT retention would be reduced by the excess bile acid production, the larger bile acid pool, and greater faecal losses.

Disordered negative feedback has many parallels in other endocrine systems when homeostatic levels are reset. For instance, in type 1 diabetes, failure to produce sufficient insulin in response to glucose results in high glucose levels. Potentially, a parallel situation could be occurring in the two patients who failed to elevate their FGF19 levels during the day despite eating. In type 2 diabetes, disordered receptor sensitivity and signaling gives higher glucose levels than expected for insulin values, and the homeostasis model assessment of insulin resistance (HOMA-IR) has gained wide acceptance [30]. A similar situation could exist in some of our patients where the median product of FGF19 and C4 is significantly higher than in normals indicating an impaired hepatic response to the negative feedback from FGF19. Parathyroid hormone secretion regulating calcium homeostasis, the hypothalamic-pituitary-adrenal axis response to stress [31], and thyroid hormone secretion are other examples of negative feedback endocrine systems that can be reset to maintain homeostasis.

Another clue to the relevance of FGF19 in primary BAM comes from earlier data that showed that FGF19 increases metabolic rate and reverses diabetes in a mouse model [32]. Deficient FGF19 results in a lower metabolic rate, and being overweight has been shown to be a highly significant association in primary BAM where the median BMIs in both men and women were about 15% higher [22].

The mechanism of the impairment in FGF19 function will need further work. Although it is clear that bile acids binding to FXR have major effects on FGF15 [24], the details of the transcriptional control of FGF15 or FGF19 (and other FXR sensitive genes such as OSTα and IBABP) in the ileum need to be clarified, together with the mechanisms responsible for

secretion of the protein. Variation in the responses of different FXR-sensitive genes in the ileum, as we have proposed [21], could be the mechanism underlying primary BAM.

Proof of the validity of this new hypothesis for the cause of primary BAM would require measurement of bile acid biosynthesis before and after FGF-19 administration, with amelioration of diarrhea corresponding to decreased bile acid biosynthesis. Measurement of bile acid output in the feces, will strengthen this further.

Confirmation of our clinical findings with FGF19 will also lead to the development of tests based on its diagnostic potential for primary BAM. We have found that it may be possible to define a fasting level of FGF19 that will discriminate between patients and most controls: a value of 146 pg/ml gives a specificity of 89%, with an overall sensitivity greater than 75%, and above 90% for patients with abnormal SeHCAT tests. These are similar to the values obtained for C4. Larger numbers in a prospective study of all patients with chronic diarrhea will refine these figures, and post-prandial values or a stimulation test may eventually prove more reliable. As the current diagnostic tests measuring C4 or SeHCAT retention both have significant problems including cost and the lack of availability in many countries such as the USA, a test based on FGF19 will have significant advantages.

There may also be a therapeutic role for FGF19, or agents that increase its production or act on its receptor, in the treatment of bile acid-induced diarrhea. Such agonists will reduce bile acid synthesis and may be more effective and acceptable than current treatments.

In summary, we have shown that a disorder of FGF19 production by the ileum can be found in patients with the condition erroneously called primary bile acid "malabsorption". Review of the existing evidence suggests that in most patients, there is no defect in bile acid absorption; however a failure to reduce bile acid secretion by negative feedback from FGF19 may occur, resulting in excess bile acids entering the colon, so causing bile acid diarrhea.

Table 1: Details of Patients and Controls

	Patients	**Controls**	***p***
n	17	19	—
M : F ratio	7 : 10	11: 8	—
Age (years) Mean ± SEM	48.1 ± 2.9	47.4 ± 4.6	NS
Bowels /24h Range Median	3 – 10 5	1 – 2 1.51	<0.0001
Previous cholecystectomy	5	0	
SeHCAT (Mean ± SEM) (% retention at day 7, n=13)	3.5 ± 0.7	—	—

Table 2: Serum FGF19, 7α-hydroxy-4-cholesten-3-one (C4) and total bile acids: Comparison between controls and different groups of patients with bile acid malabsorption

Patient Group	**n**	**FGF19** (pg/ml)	**C4** (ng/ml)	**Total BA** (μmol/l)
Control group	19	231	18	1.9
Overall diarrhea group	17	120 [b]	51 [c]	1.9
With low SeHCAT	13	118 [c]	51 [c]	1.9
Cholecystectomy	4	128 [a]	50 [b]	1.5
No cholecystectomy	9	104 [c]	65 [c]	2.3
Ileal resection group	5	8 [b]	351 [c]	1.0

Median values are shown for the groups and sub-groups. Significant differences from the control group are shown: [a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.001$ (Mann-Whitney U-test).

FIGURE LEGENDS:

Figure 1.

Fasting serum values in patients and controls of **(A)** 7α-hydroxy-4-cholesten-3-one (C4) and **(B)** Fibroblast Growth Factor 19 (FGF19). Individual values and box and whisker plots with medians, quartiles (boxes) and 5 and 95 percentiles (lines) are shown. In the patient group, solid circles indicate patients who had low SeHCAT tests and open circles those without SeHCAT tests.

Figure 2.

Inverse relationship of fasting values of serum Fibroblast Growth Factor 19 (FGF19) and 7α-hydroxy-4-cholesten-3-one (C4) in patients (solid circles) and controls (open circles).

Figure 3.

Changes during the day in six patients in serum values of **(A)** 7α-hydroxy-4-cholesten-3-one (C4) and **(B)** Fibroblast Growth Factor 19 (FGF19). Three patients had undergone cholecystectomy (open symbols) and three had not (solid symbols). Two patients (shown by the solid square and the solid triangle symbols) had no increase in FGF19 during the day. The arrows show where breakfast and lunch was eaten after taking the 9:00 and 12:00 samples.

Figure 4.

Changes in the enterohepatic circulation of bile acids that are proposed to cause primary bile acid malabsorption diarrhea. **(A)** Normal **(B)** primary BAM. The width of the lines and arrows indicate the amounts of FGF19 (black), stool (dark grey) or BA (mid-grey). Synthesis of bile acids occurs in the liver from cholesterol (Chol) by the action of CYP7A1, forming C4 and then BA. Secretion of BA into the intestine is shown schematically, and the dotted line inset demonstrates absorption in the ileum, where BA act on FXR to stimulate FGF19 production. FGF19 enters the portal venous circulation and inhibits CYP7A1 action. In (B), less FGF19 is produced in the ileum, so CYP7A1 action is greater, producing more BA. Normal BA absorption occurs in the ileum, but more BA are left unabsorbed and enter the colon, where increased secretion occurs, causing increased stool volume.

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Figure 1

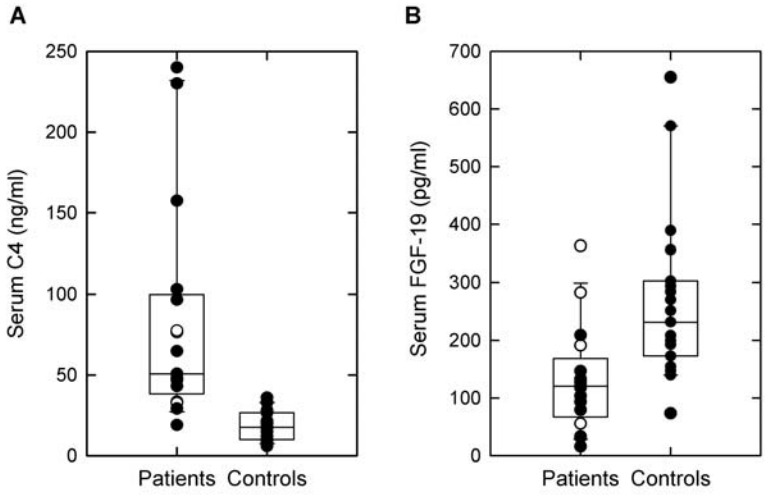

Figure 2

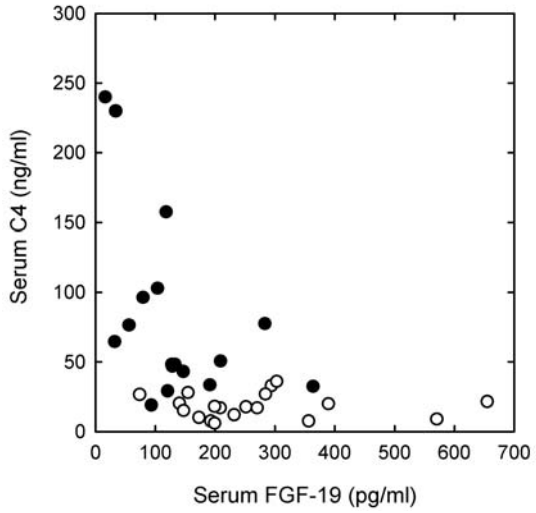

Figure 3

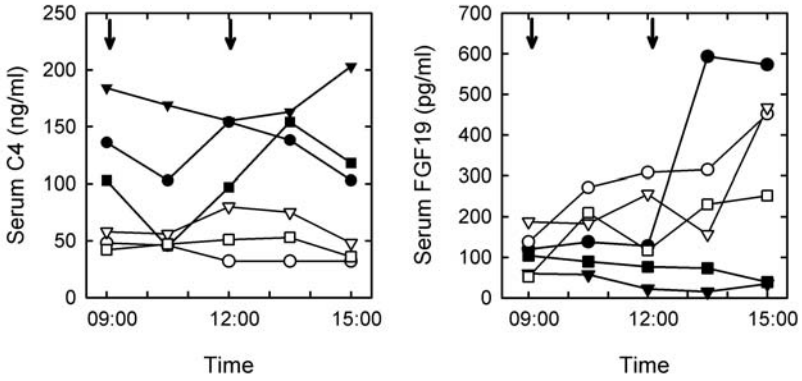

Figure 4